Proceedings of the 2nd International IEEE EMBS
Conference on Neural Engineering
Arlington, Virginia · March 16 - 19, 2005

MeaBench: A toolset for multi-electrode data acquisition and on-line analysis

Daniel Wagenaar
Caltech, Dept. of Physics
Caltech 103-33
Pasadena, CA 91125 USA

Thomas B. DeMarse
University of Florida, Dept. of BME
P.O. Box 116131
Gainesville, FL 32611 USA

Steve M. Potter
Georgia Tech, Dept. of BME
313 Ferst Dr
Atlanta, GA 30332 USA

***Abstract*—We present a software suite, MeaBench, for data acquisition and online analysis of multi-electrode recordings, especially from micro-electrode arrays. Besides controlling data acquisition hardware, MeaBench includes algorithms for real-time stimulation artifact suppression and spike detection, as well as programs for online display of voltage traces from 60 electrodes and continuously updated spike raster plots. MeaBench features real-time output streaming, allowing easy integration with stimulator systems. We have been able to generate stimulation sequences in response to live neuronal activity with less than 20 ms lag time. MeaBench is open-source software, and is available for free public download at http://www.its.caltech.edu/~pinelab/wagenaar/meabench.html.**

I. INTRODUCTION

Recording from large numbers of electrodes has become increasingly common in neuroscience over the last 30 years. Micro-electrode arrays (MEAs) [1, 2, 3] with 60 or more electrodes have been used to study many *in vitro* preparations including cortical cultures (e.g. [4, 5, 6, 7]), spinal cord cultures (e.g. [8]) as well as intact retina (e.g. [9]), while silicon probes [10] and multiwire probes (e.g. [11]) have been used extensively *in vivo*. Most labs have used commercial recording systems, which ship with dedicated software. Such software is typically not user-extendible, and not well suited for real-time applications involving bidirectional communication between computers and biological tissue. For our research on learning in embodied cultures [5, 12, 13], both are critical requirements. Here we present a software suite that fills these needs. MEABENCH is a free, open-source, set of programs for multi-electrode data acquisition (DAQ) and online analysis. MEABENCH is different from previously described multi-electrode data analysis software such as MeaTools [14], in that it directly communicates with DAQ hardware as well as providing real-time visualization. This makes it especially suitable for online operation. Thanks to its ability to communicate in real-time with stimulator hardware it can be used in closed-loop stimulation experiments.

II. METHODS

A. Software engineering

MEABENCH was programmed as a set of semi-independent programs sharing a common library. These programs communicate through standard Linux[1] inter-process communication (IPC) facilities such as pipes and shared memory. This loose modular approach was adopted to help make the software easily maintainable. It also makes it easy for third parties to add their own MEABENCH programs, e.g. to add new visualization or data export methods. The core of MEABENCH was coded in C++. Some utility programs were written in perl, and several additional data analysis tools were written for Matlab (The Mathworks, Natick, MA).

Data acquisition MEABENCH has a modular interface for communication with data acquisition (DAQ) cards. Currently, a module to acquire data from the 'MC_Card' hardware (MultiChannel Systems, Reutlingen, Germany) is well-supported. This module uses DMA transfer to minimize CPU load.

Inter-process communication MEABENCH programs use a client-server model to transfer data. A server creates a shared memory block with a header describing its contents. Clients can then independently read from this memory. To obviate the need for clients to continuously poll to check whether data are available, servers notify clients through pipes when new data become available, and when a run starts or ends. This model is used to transfer both electrode voltage data and spike information.

Multi-computer setup More advanced and more demanding algorithms for data analysis become available every year. Therefore computer hardware needs to be upgraded regularly to keep up. Yet, it is not attractive to replace a working DAQ setup, since getting DAQ hardware to work in new computers can be highly nontrivial. Therefore, MEABENCH provides the option of using one dedicated computer just for data acquisition, and a second computer for all analysis tasks. This second computer can then be upgraded whenever faster processing is required, without upsetting the DAQ computer. The two computers communicate through a (local) ethernet network.

Visualization Online data visualization is a main feature of MEABENCH. To show acquired voltage traces and spike rasters online, a set of widgets was written for use with the (open source) Qt library (Trolltech, Oslo, Norway). Qt-Designer was used to create graphical user interfaces (GUIs) for visualization programs.

This work was partially supported by grants NS044134 and NS38628 from NIH-NINDS, and EB000786 from NIH-NIBIB, and by the Burroughs-Wellcome Fund and the Whitaker Foundation.

[1] MEABENCH was designed primarily for Linux. It should work on other Unix-like operating systems with minimal changes. In particular, a port for Mac OS X (Apple Computer, Cupertino, CA) is available (from TBD, tdemarse@bme.ufl.edu).

Data streaming MEABENCH provides an interface for external programs to read data directly from MEABENCH shared memory. Additionally, MEABENCH comes with a program to output data as a posix stream, which can then be 'piped' to user's programs using standard posix shell facilities.

B. Algorithms

MEABENCH includes programs for the suppression of line noise and stimulation artifacts, as well as for spike detection. Matlab code for (multi-electrode) burst detection is included as well. The algorithms implemented by these programs are described in the following.

Line suppression Pickup at 60 Hz (or, outside the USA, at 50 Hz) is a common problem for electrophysiology. Even with careful hardware design, some pickup is usually present in micro-electrode recordings. We used an adaptive template filter to suppress this pickup digitally. This approach works best if a synchronization signal is provided from the mains on a dedicated DAQ channel. The line suppressor divides the period of the pickup into 128 time bins. A separate template of 128 values is maintained for each electrode channel. Templates are updated continuously, with a user-controlled decay time constant (typical value: 1.5 s). Subtraction of the template from the recording yields a signal cleaned of the 60 Hz line pickup and all its harmonics.

Stimulation artifact suppression Since stimulation pulses for micro-electrode arrays typically have amplitudes around 0.5 V [15], and recorded spike waveforms typically reach at most 100 μV, stimulation artifacts often occur even with carefully designed recording hardware. MEABENCH incorporates an algorithm for stimulation artifact suppresion that locally fits low-order polynomials to the recording, and subtracts the fit results [16]. This removes most artifacts that do not saturate the input stage of the recording system.

Spike detection After removal of artifacts, detecting spikes (action potentials) is often the next step in the analysis of multi-electrode recordings. To facilitate online operation, we opted for a simple amplitude-threshold detector. Thus a detection threshold that optimizes detection efficiency while controlling the rate of false positives must be established. In many cases it is not possible to temporarily switch off the biological signal, so noise levels must be estimated from the recorded superposition of signal and noise. An extra confounding factor is that noise levels often drift on a time-scale of hours, so for long-term recordings, estimates must be adapted continuously.

MEABENCH implements the following algorithm for detecting spikes. First, the recorded voltage trace is band-pass filtered between 100 Hz and 3 kHz. Then, the data stream is split into 10 ms windows, and the 2$^{\text{nd}}$ and 30$^{\text{th}}$ percentiles of the distribution of voltages in each such window are determined. Call these $V_{.02}$ and $V_{.30}$. (Note that both are usually negative because of the filtering, which sets $V_{.50} \sim \langle V \rangle \sim 0$.) Then, two tests are performed:

- Is the ratio of $V_{.02}$ over $V_{.30}$ less than 5?
- Is the absolute value of $V_{.30}$ (significantly) non-zero?

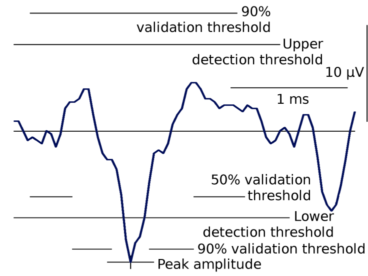

Fig. 1: Spike validation based on waveform shape. A putative spike is rejected if its waveform crosses any of the validation threshold lines, which are based on the spike's peak amplitude.

The first test makes sure that there was no actual spike in the window; the second test makes sure that the data in the window was not blanked out (e.g. by artifact removal filters). If both tests are passed, the window is considered 'clean', and $V_{.02}$ is used to update the current noise level estimate. Specifically, the noise level estimate is the output of passing the absolute values of $V_{.02}$ from all 'clean' windows through a low-pass filter with a time constant of 100 windows (i.e. 1 s if all are clean). Spikes are detected whenever the absolute value of the voltage exceeds the current noise estimate by a user-settable factor.

This algorithm adapts rapidly to changing noise situations, while not desensitizing during bursts of spikes.

Spike validation Spike waveforms are often multi-phasic, so some additional processing must be performed to prevent double detections of unitary events. We accept a spike only if its detected peak is the highest peak of either polarity within a $\pm$ 1 ms window, and no secondary peaks of the same polarity and more than 50% of the amplitude of the detected peak exists within the same window (P. P. Mitra, personal communication; see Fig. 1). This validation step also strongly reduces the rate of false positive detections.

Burst detection Culture-wide bursts or waves are observed in multi-electrode recordings from many neuronal preparations. To detect such 'global' bursts, as well as local bursts which may be of interest to researchers, MEABENCH includes an algorithm (implemented in Matlab) which first detects bursts on individual electrodes, and then groups together bursts that overlap in time. To aid in the following discussion, the term *burstlet* will be used to refer to a burst on an individual electrode. A burstlet is not necessarily a true single-electrode event: it may well co-occur with burstlets on other electrodes. A group of temporally overlapping burstlets will be called a *burst*. Bursts may be array-wide bursts, or more localized events, even including single cell bursts.

Let f_c be the average firing rate on electrode c, that is, the total number of spikes recorded on that electrode divided by the duration of the recording. We then define threshold inter-spike intervals (ISI), τ_c, for each electrode c. This τ_c is set to $\frac{1}{4f_c}$ or to 100 ms, whichever is smaller. The factor four ensures that only spikes that succeed each other faster than four times the average firing rate can be considered burstlets.

Initially, the algorithm considers each electrode independently. For a given electrode, it searches for sequences of four or more spikes with all internal ISIs less than τ_c. After these 'core' burstlets have been found, they are extended into the past and the future to also contain spikes that have ISIs less than 200 ms, (or less than $\frac{1}{3f_c}$, whichever is smaller). Thus, a burstlet consists of a core of at least four very closely spaced spikes, with an 'entourage' of any number of slightly less closely spaced spikes, all on one electrode. Once all burstlets on all electrodes have been found, they are sorted in temporal order. A burst is then simply a sequence of one or more burstlets that have non-zero temporal overlap.

In many cases, a small number of electrodes record strongly elevated firing rates for extended periods after a global burst, sometimes until the next one. If that happens, several global bursts would all be grouped together according to the algorithm as described so far. This problem is corrected in a post-processing stage. Each detected burst is considered in turn, and a graph of the number of simultaneous burstlets vs. time is constructed. If a putative burst corresponds to several global bursts, this graph will have more than one hump. The algorithm finds these humps, and splits the bursts accordingly.

C. Application

We have used MEABENCH to acquire and analyze multi-electrode array (MEA) recordings from dissociated cultures of cortical neurons. Dense cultures of cortical neurons from embryonic (E18) rats were prepared on MEAs as described before [17, 7]. Very briefly, we used MEAs with sixty 30 μm diameter electrodes spaced at 200 μm (MultiChannelSystems) in an 8x8 grid with missing corners. MEAs were precoated with poly-ethylene-imine and laminin. Cortices were dissociated using papain and trituration. Cells (both neurons and glia) were plated at a density of 2,500 cells/mm^3. Cultures were maintained in a DMEM-based medium [18] in Teflon-sealed dishes [17] in an incubator with 65% relative humidity. This low humidity made the incubator safe for electronics, allowing us to perform all recordings inside the incubator. The Teflon seals prevented evaporation. Partly replacing media every 5–7 days, we could maintain cultures indefinitely.

Electrode signals were amplified and digitized using Multi-Channel Systems hardware, controlled by MEABENCH. For stimulation, we used a custom device [19] which connects to the MultiChannel Systems pre-amplifier, and which can generate arbitrary spatio-temporal stimulation sequences under real-time control.

Use of TTX for noise estimation To obtain an unbiased estimate of the recording noise, we bath-applied 1 μM tetrodotoxin (TTX) to a culture and recorded 300 s of voltage traces after equilibration. By feeding these traces to the spike detector, we determined the rate of false positive spikes[2] (per second, per recording channel) as a function of the detection threshold. By subtracting this number of spikes from the firing rate in the active culture (recorded before TTX application), we could estimate the true firing rate, and hence the detector efficiency as a function of the detection threshold.

[2] While bathed in TTX, neurons cannot generate sodium action potentials, so any detected spikes must necessarily be false positives.

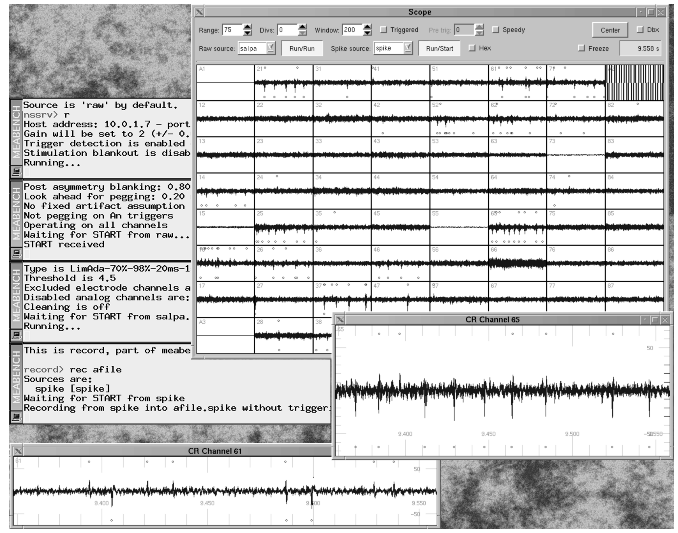

Fig. 2: Screenshot of MEABENCH in operation. The large window displays voltage traces from all electrodes, in MEA geometry. Small windows on the side show the command-line interfaces of (top to bottom) Rawsrv, Salpa, Spikedet, and Record.

III. RESULTS

A. Programs

MEABENCH consists of the following main programs:

- Rawsrv — The grandmother server. It reads voltage traces from the hardware and makes them available to other MEABENCH programs.
- Spikedet — A threshold-based spike detector. It reads from a voltage-trace stream, and publishes a spike information stream. Includes algorithms to adapt to fluctuating noise levels.
- 60hz — Template filter to remove 60 Hz pickup.
- Salpa — Stimulation artifact filter [16].
- Record — Records voltage or spike data to disk.
- Replay — Replays files created by Record.
- Scope — GUI program for online display of voltage and spike data. Scope includes a 'freeze' feature for instant-replay of the last 5 s of data.
- Spikesound — GUI program for online sonification of spike data.
- Flexraster — GUI program for online generation of spike raster plots. Flexraster allows zooming and scrolling through an entire recording.
- Neurosock and Nssrv — An alternative to Rawsrv that allows one to dedicate one computer to data acquisition, and another for online analysis (see Methods).

In addition, a number of scripts are provided for off-line processing and for automating data acquisition tasks. Full details may be found in the MEABENCH User Guide (included with the software). An example MEABENCH session is depicted in Fig. 2.

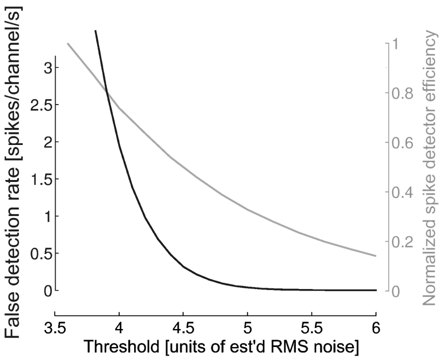

Fig. 3: Tradeoff between spike detector efficiency and rate of false positive detection.

B. Artifact suppression

Stimulation artifacts were suppressed within 1 ms after the recording system returned from saturation, allowing spike detection within 2 ms after stimulation on most electrodes [16]. (Typically, the stimulated electrode remained saturated for 50–100 ms.)

C. Spike detection

What constitutes an acceptable rate of false positive detections typically depends on the experimental situation. To be able to make an educated decision about appropriate spike detector threshold settings, we determined the detection rate of false positives in a culture quieted by TTX (see Methods). If one false positive per second per channel is acceptable, the detection threshold could be set at 4.25x estimated RMS noise (Fig. 3). Increasing the detection threshold reduced the rate of false positive detections, but also reduced the detector efficiency.

D. Real time operation and stimulator control

A typical MEABENCH online data processing chain consisting of stimulation artifact suppression, spike detection, recording, and visualization could be run on a Linux system with an AMD Athlon XP 2800+ CPU. Artifact suppression took about 20% of CPU time, spike detection 10%, and recording about 3%. Thus, sufficient CPU power was available to display continuous voltage traces at a 5 Hz frame rate, as well as continuously updated raster plots.

Thanks to the modular structure of MEABENCH, it required only a few lines of perl code to make it control our custom stimulator [19]. In this way, we could generate stimulation sequences as a function of observed neuronal activity in real-time, with less than 20 ms latency between activity and generated stimuli[3]. Thus, MEABENCH allowed real-time bidirectional communication with neuronal cultures. Recently, we used this to control and suppress culture-wide bursting by providing stimulation controlled by a real-time feedback loop to maintain a stable tonic firing rate [7].

[3] Using a special low-latency version of the Linux kernel. With standard Linux, the latency was about 50 ms.

IV. DISCUSSION

MEABENCH was first conceived to facilitate the study of learning in embodied neuronal networks [5]. We needed a data acquisition system that could communicate in real-time with arbitrary code to transform outputs from a neuronal culture to motor commands for a simulated animal (animat) or robot. MEABENCH has since developed into an extensive and very stable framework for multi-electrode data acquisition and analysis, and has become a core component of our experimental setups. In our labs, MEABENCH is used in conjunction with MEAs, but its modular structure makes it straightforward to adapt it to different hardware, including *in vivo* multi-electrode probes. MEABENCH is available for free public download at http://www.its.caltech.edu/∼pinelab/wagenaar/meabench.html.

ACKNOWLEDGMENT

We thank Jerry Pine for many useful discussions, and Sheri McKinney for assistance with animal handling and culture preparation.

REFERENCES

[1] C. A. Thomas, Jr, P. A. Springer, G. E. Loeb, Y. Berwald-Netter, and L. M. Okun, "A miniature microelectrode array to monitor the bioelectric activity of cultured cells," *Exp. Cell. Res.*, vol. 74, pp. 61–66, 1972.

[2] G. W. Gross, "Simultaneous single unit recording in vitro with a photoetched laser deinsulated gold multimicroelectrode surface," *IEEE Trans. Biomed. Eng.*, vol. 26, no. 5, pp. 273–279, 1979.

[3] J. Pine, "Recording action potentials from cultured neurons with extracellular microcircuit electrodes," *J. Neurosci. Methods*, vol. 2, no. 1, pp. 19–31, 1980.

[4] Y. Jimbo, T. Tateno, and H. P. C. Robinson, "Simultaneous induction of pathway-specific potentiation and depression in networks of cortical neurons," *Biophys. J.*, vol. 76, no. 2, pp. 670–678, 1999.

[5] S. M. Potter, "Distributed processing in cultured neuronal networks," in *Progress in Brain Research*, M. A. L. Nicolelis, Ed., vol. 130, pp. 49–62. Elsevier Science, 2001.

[6] G. Shahaf and S. Marom, "Learning in networks of cortical neurons," *J. Neurosci.*, vol. 21, no. 22, pp. 8782–8788, 2001.

[7] D. A. Wagenaar, R. Madhavan, J. Pine, and S. M. Potter, "Controlling bursting in cortical cultures with closed-loop multi-electrode stimulation," *J. Neurosci.*, 2005, in press.

[8] G. W. Gross and J. M. Kowalski, "Origins of activity patterns in self-organizing neuronal networks in vitro.," *J. Intell. Mater. Syst. Struct.*, vol. 10, no. 7, pp. 558–564, 1999.

[9] M. Meister, J. Pine, and D. A. Baylor, "Multi-neuronal signals from the retina – acquisition and analysis," *J. Neurosci. Methods*, vol. 51, no. 1, pp. 95–106, 1994.

[10] K. D. Wise and J. B. Angell, "Low-capacitance multielectrode probe for use in extracellular neurophysiology," *IEEE Trans. Biomed. Eng.*, vol. 22, no. 3, pp. 212–219, 1975.

[11] M. A. L. Nicolelis, A. A. Ghazanfar, B. M. Faggin, S. Votaw, and L. M. O. Oliveira, "Reconstructing the engram: Simultaneous, multisite, many single neuron recordings," *Neuron*, vol. 18, no. 4, pp. 529–537, 1997.

[12] D. J. Bakkum, A. C. Shkolnik, G. Ben-Ary, P. Gamblen, P. B. DeMarse, and S. M. Potter, "Removing some 'A' from AI: Embodied cultured networks," in *Embodied Artificial Intelligence*, F. Iida, L. Steels, and R. Pfeifer, Eds. Springer-Verlag, 2004, in press.

[13] S. M. Potter, D. A. Wagenaar, and T. B. DeMarse, "Closing the loop: Stimulation feedback systems for embodied MEA cultures," in *Advances in network electrophysiology using multi-electrode arrays*, M. Taketani and M. Baudry, Eds. Kluwer, New York, 2004, in press.

[14] U. Egert, T. Knott, C. Schwarz, M. Nawrot, A. Brandt, S. Rotter, and M. Diesmann, "MEA-Tools: an open source toolbox for the analysis of multi-electrode data with MATLAB," *J. Neurosci. Methods*, vol. 117, no. 1, pp. 33–42, 2002.

[15] D. A. Wagenaar, J. Pine, and S. M. Potter, "Effective parameters for stimulation of dissociated cultures using multi-electrode arrays," *J. Neurosci. Methods*, vol. 138, no. 1-2, pp. 27–37, 2004.

[16] D. A. Wagenaar and S. M. Potter, "Real-time multi-channel stimulus artifact suppression by local curve fitting," *J. Neurosci. Methods*, vol. 120, no. 2, pp. 113–120, 2002.

[17] S. M. Potter and T. B. DeMarse, "A new approach to neural cell culture for long-term studies," *J. Neurosci. Methods*, vol. 110, no. 1-2, pp. 17–24, 2001.

[18] Y. Jimbo, H. P. C. Robinson, and A. Kawana, "Strengthening of synchronized activity by tetanic stimulation in cortical cultures: Application of planar electrode arrays," *IEEE Trans. Biomed. Eng.*, vol. 45, no. 11, pp. 1297–1304, 1998.

[19] D. A. Wagenaar and S. M. Potter, "A versatile all-channel stimulator for electrode arrays, with real-time control," *J. Neural Eng.*, vol. 1, pp. 39–44, 2004.